National Toxicology Program
Toxicity Report Series
Number 66

NTP Technical Report
on the Toxicology Studies of

3,3′,4,4′-Tetrachloroazoxybenzene

(CAS No. 21232-47-3)

Administered by Gavage to
F344/N Rats and B6C3F$_1$ Mice

Angélique P.J.M. van Birgelen, Ph.D., Study Scientist
National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. Department of Health And Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

National Toxicology Program
Toxicity Report Series
Number 66

NTP Technical Report
on the Toxicity Studies of

3,3′,4,4′-Tetrachloroazoxybenzene

(CAS No. 21232-47-3)

Administered by Gavage
to F344/N Rats and B6C3F$_1$ Mice

Angélique P.J.M. van Birgelen, Ph.D., Study Scientist
National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

A.P.J.M. van Birgelen, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
C.D. Hébert, Ph.D.
J. Mahler, D.V.M.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems

Microbiological Associates, Inc.

Conducted studies, evaluated pathology findings

M.L. Wenk, Ph.D., Principal Investigator
R.M. Kovatch, D.V.M.
L.L. Pippin, D.V.M.
J.M. Pletcher, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, D.V.M., M.S., Ph.D.

Environmental Health Research and Testing, Inc.

Provided sperm motility and vaginal cytology evaluations

L.K. Grimes, D.V.M., Principal Investigator
T.A. Sexton

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats and mice (25 September 1996)

D.G. Goodman, V.M.D., Chairperson
PATHCO, Inc.
N. Barlow, D.V.M., Observer
North Carolina State University
S. Botts, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
D. Wolf, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
K.P. McGowan, M.B.A.
M.A. Mauney, M.S.
N.G. Mintz, B.S.
J.T. Scott, M.S.

Biotechnical Services, Inc.

Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
J.R. Carlton, B.A.
A.M. Macri-Hanson, M.A., M.F.A.
W.D. Sharp, B.A., B.S.
S.M. Swift, B.S.

PEER REVIEW

The draft report on the toxicity studies of 3,3′,4,4′-tetrachloroazoxybenzene was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

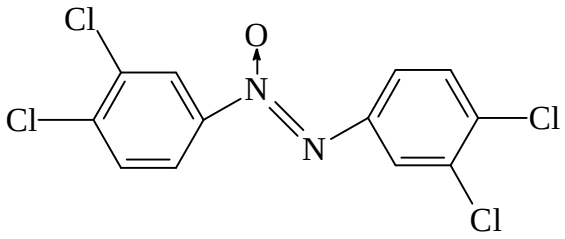
Abraham Brouwer, Ph.D.
Division of Toxicology
Department of Food Technology
and Nutritional Sciences
Agricultural University Wageningen
Wageningen, The Netherlands

John M. Cullen, Ph.D., V.M.D.
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

John P. Geisy, Ph.D.
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI

CONTENTS

ABSTRACT



3,3′,4,4′-TETRACHLOROAZOXYBENZENE

CAS No. 21232-47-3

Chemical Formula: $C_{12}H_6Cl_4N_2O$ Molecular Weight: 336.0

Synonyms: Azoxybenzene, 3,3′,4,4′-tetrachloro-(8CI); diazene, bis(3,4-dichlorophenyl)-1-oxide-(9CI); TCAOB

3,3′,4,4′-Tetrachloroazoxybenzene is not commercially manufactured but is present as a contaminant of 3,4-dichloroaniline and its herbicidal derivative Diuron®. In addition, environmental contamination occurs when 3,3′,4,4′-tetrachloroazoxybenzene is formed by the photolysis and biolysis of 3,4-dichloroaniline. 3,3′,4,4′-Tetrachloroazoxybenzene was nominated by the United States Environmental Protection Agency for toxicity testing based on concerns over the potential for human exposure, the structural resemblance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and the reported dioxin-like effects of 3,3′,4,4′-tetrachloroazoxybenzene. The toxicity of 3,3′,4,4′-tetrachloroazoxybenzene was evaluated in 16-day and 13-week gavage studies in male and female F344/N rats and B6C3F_1 mice. In addition to histopathology, evaluations included hematology (rats only), clinical chemistry, thyroid hormone analyses (rats only), hepatic cell proliferation (rats only), cytochrome P_{450}1A immunohistological staining in the liver (rats only), and assessments of male reproductive endpoints and estrous cycle length. Additional genetic toxicology studies included mutagenicity tests in *Salmonella typhimurium* and the determination of micronuclei in mouse bone marrow and peripheral blood erythrocytes.

In the 16-day studies, groups of five male and five female rats received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage at doses of 0, 12.5, 32, 80, 200, or 500 mg per kg body weight, 5 days a week. Groups of five male and five female mice received 0, 1, 3.2, 10, 32, or 100 mg/kg in corn oil by gavage, 5 days a week. Major effects in rats included increases in liver and lung weights, and decreases in mean body weights and body

weight gains, heart weights, and thymus weights. Effects in mice included increases in liver weights and decreases in thymus weights. No effects on survival were observed. Treatment-related lesions included cytoplasmic alteration of hepatocytes, splenic hematopoietic cell proliferation, thymic atrophy, and nephropathy in rats and thymic atrophy, splenic hematopoietic cell proliferation, and hepatic foci of inflammation and necrosis in mice.

In the 13-week studies, groups of 10 male and 10 female rats and mice received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage at doses of 0, 0.1, 1, 3, 10, or 30 mg/kg, 5 days a week.

In the 13-week rat study, all males and seven females in the 30 mg/kg groups died. Decreases in final mean body weights and body weight gains were observed in 3 and 10 mg/kg males and 10 and 30 mg/kg females. Decreased thymus weights, accompanied by thymic atrophy observed microscopically, were observed at doses of 1 mg/kg or greater in males and females. Increased liver weights were observed in males and females administered 1 mg/kg or greater, and hepatic cytochrome P_{450}1A staining was increased in 1 and 3 mg/kg males and 3, 10, and 30 mg/kg females. In addition, a responsive anemia and decreases in platelet counts were observed in dosed male and female rats. A marked decrease in circulating thyroxine concentrations was observed in dosed males and females. In spite of this sharp decrease, thyroid-stimulating hormone concentrations were marginally increased. A decrease in epididymal spermatozoal motility was observed in all dosed groups tested. In 10 mg/kg females, the estrous cycle length was increased. Major effects included increased incidences of hyperplasia of the forestomach in 3, 10, and 30 mg/kg males and 10 and 30 mg/kg females. Increased incidences of centrilobular degeneration and hematopoietic cell proliferation were observed in the liver of dosed males and females. Furthermore, chronic active inflammation of the lung vasculature and hematopoietic cell proliferation in the spleen were observed in dosed males and females. The increased severities of cardiomyopathy and nephropathy in males and the incidences of cardiomyopathy and nephropathy and severity of cardiomyopathy in females were 3,3′,4,4′-tetrachloroazoxybenzene related.

In the 13-week mouse study, the major effects included increases in liver weights in males administered 3 mg/kg or greater and females administered 1 mg/kg or greater. Hyperplasia of the forestomach and dilatation of hair follicles were observed in 10 and 30 mg/kg males and 30 mg/kg females. Furthermore, thymus weights were decreased in males administered 3 mg/kg or greater and in 10 and 30 mg/kg females. Increased incidences of centrilobular hypertrophy of hepatocytes were observed in 10 and 30 mg/kg males and females. Increased incidences of hematopoietic cell proliferation in the spleen were observed in 30 mg/kg males and in 10 and 30 mg/kg females. Increases in the incidences of thymocyte necrosis were observed in 10 mg/kg males and in 10 and 30 mg/kg females. The incidences of splenic pigmentation were increased in all dosed groups of males, and the severity of pigmentation increased with increasing dose in males and females.

3,3′,4,4′-Tetrachloroazoxybenzene was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535 with or without induced S9 metabolic activation enzymes. It did not induce significant increases in micronucleated erythrocytes in a three-exposure male mouse bone marrow micronucleus test up to dose levels of 200 mg/kg, but results of a 13-week peripheral blood micronucleus test conducted in male and female mice were positive.

In summary, 3,3′,4,4′-tetrachloroazoxybenzene caused typical dioxin-like effects, including thymic atrophy, increased liver weights, induction of hepatic cytochrome P_{450}1A, and decreased mean body weight gains. Furthermore, a marked decrease in circulating thyroxine concentrations was observed in male and female rats, even at the lowest dose (0.1 mg/kg) in female rats. A decrease in epididymal sperm motility was observed at all doses in rats. Effects on the hematopoietic system occurred at doses including and lower than those that caused histopathologic alterations in the liver. A no-observable-adverse-effect-level (NOAEL) was not reached in rats. In male and female mice, the NOAEL was 1 and 0.1 mg/kg, respectively. Furthermore, treatment-related effects included increased incidences of hyperplasia of the forestomach epithelium in rats and mice, chronic active inflammation of the vasculature of the lung in rats, increased incidences and/or severities of cardiomyopathy and nephropathy in rats, and dilatation of the hair follicles in mice. Comparison of various dioxin-like effects in these studies with those reported in the literature indicate that 3,3′,4,4′-tetrachloroazoxybenzene is six to two orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.